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Fructosamine: Structure, Analysis, and Clinical Usefulness

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Glucose molecules are joined to protein molecules to form stable ketoamines, or fructosamines, through glycation, a nonenzymatic mechanism involving a labile Schiff base intermediate and the Amadori rearrangement. The amount of fructosamine in serum is increased in diabetes mellitus owing to the abnormally high concentration of sugar in blood. The concentration of fructosamine in serum thus reflects the degree of glycemic control attained by the diabetic patient and is useful in monitoring the effectiveness of therapy in diabetes over a period of several weeks, in a manner analogous to the determination of glycated hemoglobin. Of the analytical approaches used to measure fructosamine, affinity chromatography with *m*-aminophenylboronic acid and the nitroblue tetrazolium reduction method appear to be the most practical means for clinical chemists to assay fructosamine quickly, economically, and accurately. Fructosamine values can readily distinguish normal individuals and diabetic patients in good glycemic control from diabetics in poor control. Unlike glycated hemoglobin, which reflects the average blood sugar concentration over the past six to eight weeks, fructosamine reflects the average blood sugar concentration over the past two to three weeks. Thus a clinical advantage is that fructosamine responds more quickly to changes in therapy, thereby allowing for improved glycemic control. Used in conjunction with determinations of blood sugar and (or) of glycated hemoglobin, or by itself, the fructosamine assay can provide clinically useful information for the detection and control of diabetes. Key words:

Introduction

Diabetes affects about 5% of the American population and is the fifth leading cause of death in the United States. Fortunately, early detection and improved therapeutic regimens allow diabetic patients to lead normal lives, and life-expectancy figures have increased. Clinical chemistry plays an integral role in the diagnosis and treatment of diabetes. The determination of blood glucose is the *sine qua non* for both detection and therapy. The assay of glycated hemoglobin, or Hb A_{1c} specifically, is a fairly recently developed test; it is now accepted and used as a means of monitoring the long-term control of blood sugar.[1] Another new test, the measurement of glycated albumin or glycated total proteins, commonly referred to as the fructosamine assay, may be used in a manner analogous to the determination of glycated hemoglobin as an index to the mean concentration of glucose in blood during the preceding several weeks. As yet, the fructosamine assay is not widely or routinely used; hence, in this review, I describe protein glycation, trace the development of the fructosamine procedures, compare and contrast the various analytical methodologies that have been applied to the assay, and examine the clinical usefulness of the test.

Nomenclature. The term "fructosamine" in this review refers to glycated albumin and protein, which have been abbreviated in the literature as GSA or GPA (glycated serum or plasma albumin) and GSP or GPP (glycated serum or plasma protein). The term "glycation" is recommended by the IUPAC-IUB Joint Commission on Biochemical Nomenclature for any reaction linking a sugar to a protein (1). Thus "glycated" protein is preferable to "glycosylated" or "glucosylated" protein, although both of these terms are common in the literature. Johnson et al. (2) introduced fructosamine into the clinical chemistry literature in 1982 as a general term for glycated protein. Because fructosamine seems to have been generally accepted in the field and because it is less cumbersome than "glycated serum protein" or similar phrases and less ambiguous than an abbreviation, I use it preferentially in this paper and interchangeably with glycated albumin and glycated protein.

Fructosamine is the trivial name for 1-amino-1-deoxy-fructose, also called isoglucosamine by Emil Fischer, who first synthesized the compound in 1886 (3, 4). More broadly, a fructosamine is a ketoamine, a derivative of the nonenzymatic reaction product of a sugar (usually glucose) and a protein (usually albumin). As noted, the terms glycated albumin or protein are commonly used in the literature and are examples of fructosamines. In contrast, a glycoprotein is a protein molecule, typically a globulin, that contains a carbohydrate moiety, which was incorporated enzymatically during the synthesis of the molecule. The fructosamines arise from a post-translational modification involving a nonenzymatic mechanism and should not be confused with the glycoproteins.

Chemistry of glycation. Maillard was the first to describe

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[1] Nonstandard abbreviations: DMF, 1-deoxy-1-morpholinofructose; Hb A_1 or Hb A_{1c}, glycated hemoglobin; NBT, nitroblue tetrazolium; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,4-diphenyltetrazolium bromide; TBA, 2-thiobarbituric acid; HMF, 5-hydroxymethyl-furfuraldehyde; TCA, trichloroacetic acid; IUPAC-IUB, International Union of Pure and Applied Chemistry–International Union of Biochemistry.

the reaction of amino acids and reducing sugars to form stable ketoamine adducts (5). The Maillard reaction is responsible for the "browning" phenomenon that occurs in milk and other food products (6-9). When milk and other foods are heated, carbonyl groups of sugars combine with the amino groups of proteins to form aldimines (Schiff bases), which progress to stable ketoamines (fructosamines); further reactions result in poorly soluble brown products. The labile aldimine–Schiff base intermediates undergo the Amadori rearrangement (the isomerization of an aldosylamine to a 1-amino-1-deoxy-2-ketose) to form the stable fructosamine (10, 11). The straight-chain fructosamine, if formed from glucose, is thought to undergo cyclization to a hemiketal furanose or pyranose ring structure for added stability (12-14).

Figure 1 shows the reaction scheme for glucose and a protein, with the formation of fructosamine.

Protein glycation. Holmquist and Schroeder (15), in 1966, reported that hemoglobin A_{1c} (Hb A_{1c}) forms a Schiff base with a ketone or aldehyde at the N-terminus of the beta chains. The Schiff base linkage is labile but can be stabilized by reduction with $NaBH_4$. The N-terminal blocking group was hypothesized to be glucose, and Hb A_{1c} was synthesized by incubating erythrocytes and hemolysates with glucose (16). Koenig et al. (17) demonstrated that glucose reacts with the N-terminal valine of hemoglobin beta chains to form 1-deoxy-1-(N-valyl)-fructose through the Amadori rearrangement. Hemoglobin glycation occurs not only at the valine residues of the beta chain termini but also nonspecifically at the epsilon amino groups of lysines along the alpha and beta chains and at the N-termini of the alpha chains (18, 19). Hemoglobin reacts with glucose and other sugars, but also with aldehydes such as formaldehyde, acetaldehyde, and glyceraldehyde (13, 20). The assay of glycated hemoglobin is now routinely used, but it is complicated by the variety of glycohemoglobins detectable, several interferences, and the number of methodologies that are used (21).

The nonenzymatic glycation of hemoglobin having been established and shown to be significantly increased in diabetes, it was logical to ask whether this was a phenomenon common to other proteins as well. Not surprisingly, many proteins have been shown to be subject to glycation, and these findings are often relevant to the complications associated with diabetes. Table 1 summarizes the proteins other than albumin and the common proteins found in the blood that undergo glycation. In 1979 glycated rat and human albumin were prepared in vitro, and glycated albumin was found to be naturally occurring in both species (59-62). Albumin was found to be glycated at multiple sites and primarily at the epsilon-amino groups of lysine residues, as is the case with hemoglobin (63). Glycation of albumin and other plasma proteins is increased in diabetes as compared with normal subjects, and the measurement of fructosamine correlates with Hb A_{1c} values. Fructosamine may thus be used in a manner similar to glycated hemoglobin to monitor the average concentration of blood glucose over an extended period of time: about one to three weeks for fructosamine and about six to eight weeks for Hb A_1 (64-66).

Analytical Approaches

At least five different methods have been used to measure fructosamine (Figure 2). Each of these will now be described, with special emphasis on those that have found favor in the clinical laboratory.

Phenylhydrazine Procedure

Acharya and Manning (67), after preparing a stable ketoamine from glyceraldehyde and hemoglobin A, reacted this fructosamine with phenylhydrazine. The resulting phenylhydrazone adduct had an absorption maximum at 350 nm and was formed in amounts directly proportional to the amount of glyceraldehyde bound to hemoglobin.

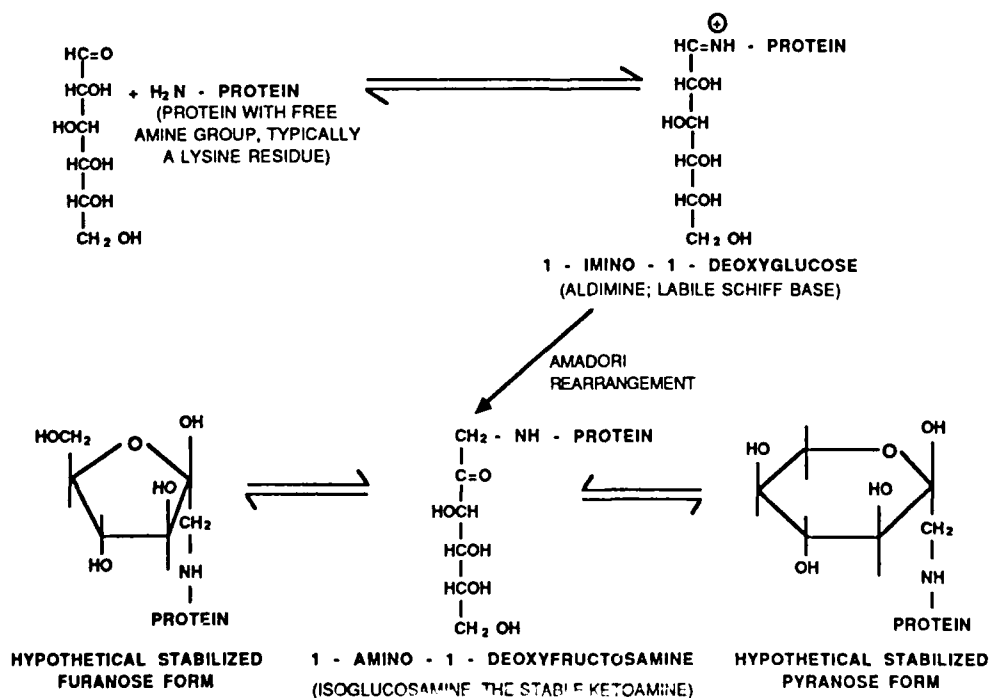Ghiggeri et al. (68) purified glycated albumin from diabetic serum and found that it reacted with phenylhydrazine to

Fig. 1. The reaction of glucose and protein to form fructosamine

Table 1. Examples of Proteins Subject to Nonenzymatic Glycation

Protein	Source	Reference
Collagen	Human tendon	22–24
Collagen	Rat aorta	25
Collagen	Rat glomerular basement membrane	26, 27
Collagen	Human glomerular basement membrane	28, 29
Collagen	Calf skin	30
Tubulin	Rat brain	31
Ocular lens proteins	Bovine ocular lenses	32
Ocular lens proteins	Rat ocular lenses	27, 32–35
Ocular lens proteins	Human ocular lenses	35–39
Peripheral nerve protein	Rat and dog sciatic nerve	40, 41
Peripheral nerve protein	Human femoral nerve	29
Tendon protein	Human digastric muscle	29
Artery protein	Human coronary artery	29
Elastin	Human lung connective tissue	29
Erythrocyte membrane protein	Human erythrocytes	42–45
Insulin	Bovine insulin preparation	46
Antithrombin III	Human plasma	47–49
Platelet protein	Human platelets	50, 51
Lipoproteins	Human high- and low-density lipoproteins	52–55
Fibrinogen	Human fibrinogen	56
Ribonuclease A	Bovine pancreatic ribonuclease A	57
Urinary amino acids and peptides	Human urine	58

form a phenylhydrazone adduct also absorbing at 350 nm. This approach does not appear to have been further developed for use in the clinical laboratory.

Furosine Procedure

The epsilon-amino group of lysine is the principal site of nonenzymatic attachment of glucose to protein molecules (63). Finot et al. (69) found that ϵ-N-(1-deoxy-D-fructosyl)-L-lysine, when subjected to hydrolysis and dehydration, produces ϵ-N-(2-furoylmethyl)-L-lysine (furosine). Schleicher et al. have quantified glycated albumin and erythrocyte membranes by measuring furosine (45, 70). Fructosamine is hydrolyzed for 18 h in 6 mol/L HCl at 95 °C to yield about 50% lysine, 30% furosine, 10% pyridosine, and other products. Furosine is quantified by liquid chromatography on a reversed-phase column with H_3PO_4 (7 mmol/L, as the mobile phase, and dual ultraviolet detection at 254 and 280 nm). Reduction with $NaBH_4$ eliminates the keto group of fructose-lysine and no furosine is found after hydrolysis, proving the specificity of the method for fructosamine. Fructose-lysine is the major ketoamine in serum proteins, but other fructose-substituted amino acids can be measured by this method if the appropriate standards are used. Although this assay is recommended by its specificity and eloquence, the 18-h hydrolysis step is probably its major drawback for routine use in the clinical laboratory; however, the chromatographic analysis could be automated.

Affinity Chromatography

Phenylboronic acid in alkaline solution complexes with the cis-diol groups of sugars. To separate fructosamines from urine, Brownlee et al. (58) developed an affinity chromatographic system that uses m-aminophenylboronic acid immo-
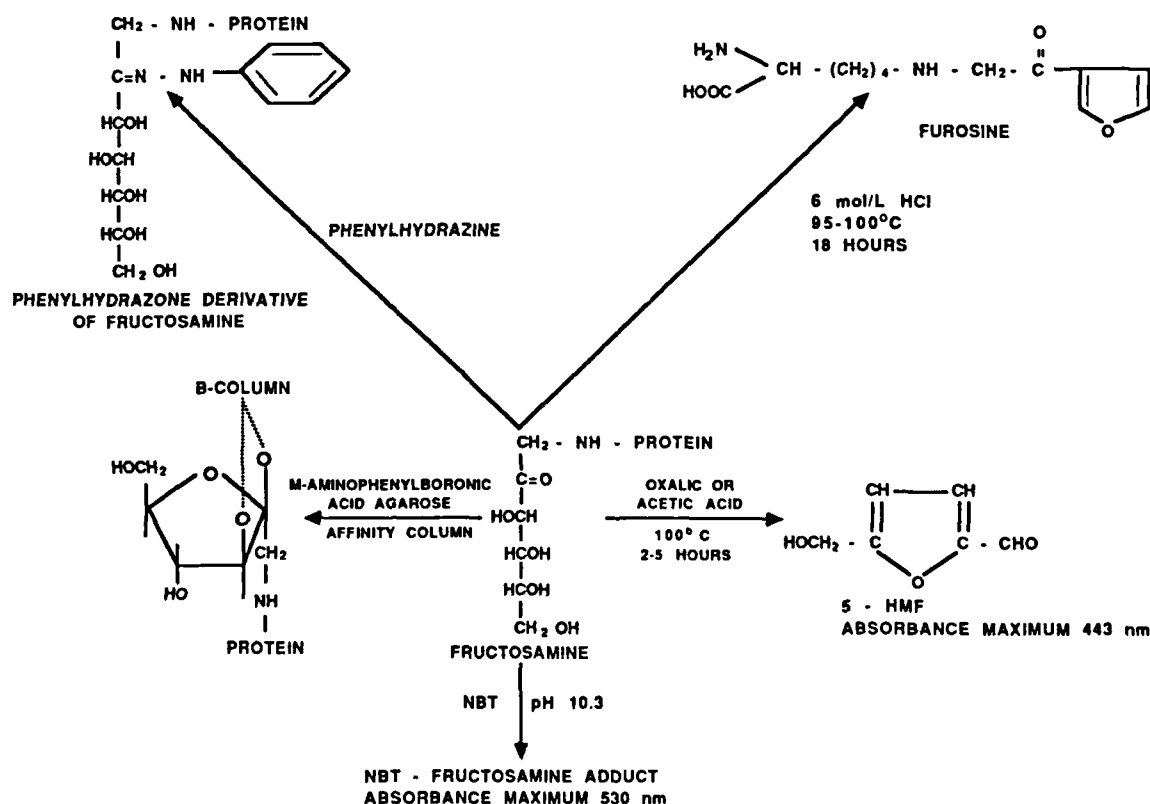

Fig. 2. Various analytical approaches to fructosamine analysis

bilized on a column. One commercial kit based on affinity chromatography ("Glyc-affin"; Isolab, Akron, OH) was evaluated by Yatscoff et al. (71) in a study of diabetic patients and normal subjects. Within-run CV's were < 6.5% for both glycated albumin and glycated protein; day-to-day CVs were 7.7% for albumin and 14.4% for protein for normal subjects and 10.5% and 17.8%, respectively, for diabetics. Temperature variation must be controlled because results decrease as the temperature increases. Free glucose interferes and must be removed, either by exclusion column chromatography or dialysis. There was no interference by labile (aldimine) fraction, lipemia, icterus, hemolysis, or anticoagulants. Yatscoff et al. (71) recommended that the albumin or total protein concentrations be standardized for improved reproducibility. Mahaffey et al. (72), using this kit to study diabetic dogs, found that concentrations of glycated albumin and protein in serum were significantly higher (P <0.001) in diabetic than in nondiabetic dogs and that the concentrations of both analytes were similar when nondiabetic and well-controlled diabetic dogs were compared. Gould et al. (73) evaluated the affinity-column method used in another commercially available phenylboronic acid gel kit ("Glycogel"; Pierce Chemical Co., Rockford, IL). This kit discriminated between normal subjects and diabetic patients, exhibited good precision (CVs of 4–6%), and results correlated with those by the thiobarbituric acid method (r = 0.70). Leiper et al. (74) also prepared aminophenylboronic acid affinity columns containing Glycogel to measure fructosamine in pregnant diabetics. Within-run CVs of 6.1% or less were obtained for both glycated albumin and protein in both diabetic and normal samples. Rendell et al. (75, 76) also assessed Glycogel affinity columns and found the method to have several advantages over the thiobarbituric acid method. Affinity chromatography appears to be simple and to give reasonably reproducible results, but is not yet widely used in clinical laboratories.

A related assay for glycated albumin, also based upon complex formation between the cis-diol groups of sugars and phenylboronic acid, has been developed by Hayashi and Makino (77). Serum is mixed with a solution of N-(5-dimethylamino-1-naphthalenesulfonyl)-3-aminobenzene boronic acid (dansylated phenylboronic acid). The mixture is excited at 330 nm, 20 C, and fluorescence emission is measured at 490 nm.

2-Thiobarbituric Acid (TBA) Colorimetric Procedure

Gottschalk (3) first discovered that treating fructosamines with acid yields 5-hydroxymethylfurfuraldehyde (HMF). HMF has been estimated by measuring its absorbance at 284 nm (78). However, the typical approach, first reported by Keeney and Bassette (7), has been to react HMF with TBA to form a derivative that has an absorbance maximum at 443 nm. The TBA procedure had been used in the analysis for Hb A₁c and was subsequently adapted for the assay of fructosamine (16, 59). Early assays typically involved reacting a purified albumin solution, serum, or plasma with acetic or oxalic acid for 18–24 h at about 100 C to hydrolyze fructosamine and convert it to HMF; after trichloroacetic acid (TCA) was added to precipitate the protein, the supernatant fluid was reacted with TBA at 40 °C for about 30 min to form an adduct measured at 443 nm (58–60, 62, 64, 65). HMF was used as the standard and results were reported as moles of HMF per mole of albumin or protein, necessitating the quantification of albumin or total protein.

To improve accuracy, investigators have introduced a dialysis step to remove free glucose, which falsely increases results, and the use of a specimen blank, in which fructosamine is reduced with NaBH₄ (66, 79, 80). As an alternative to the 12- to 24-h dialysis step to remove endogenous glucose, one can precipitate the proteins, including fructosamine, with TCA before the hydrolysis step (81). Murtiashaw et al. (82) developed a similar modification, but used ethanol rather than TCA for protein precipitation and also incorporated a differential absorbance measurement to eliminate the sample blank.

Dolhofer and Wieland (83) made concerted efforts to improve the TBA assay and determined that: (a) the amount of NaBH₄ used influences results, (b) the protein concentration should be standardized in all samples, (c) both glucose and NaBH₄ should be removed before the hydrolysis, and (d) hydrolysis with acetic acid yields higher absorbances than does hydrolysis with oxalic acid. Ney et al. (84) proposed optimal, standardized conditions that include hydrolysis at 115 C in an oil bath for 8 h, a 50-min incubation with TBA, and glycated albumin standards. Elder and Kennedy (85) suggested three modifications to optimize the assay: (a) incubating the serum blanks with NaBH₄ for only 15 min, (b) performing the hydrolysis step in an autoclave at 121 C, and (c) using fructose rather than HMF standards. These changes decrease the analysis time to about 2–5 h instead of 10 h, starting with dialyzed serum. The TBA assay has been adapted to the Technicon AutoAnalyzer II as a semi-automated method (86, 87).

Clearly, the TBA method has undergone considerable development with a number of variations extant. Its major drawbacks are the typically long analysis time and the number of steps involved. Dialysis of specimens or protein precipitation to remove interfering endogenous glucose, preparation of NaBH₄-reduced serum blanks, the hydrolysis step, and the long incubation with TBA combine to make it a labor- and time-intensive procedure. To measure glycated albumin specifically, it must be separated from the other serum proteins, further complicating the procedure. The use of a molecular exclusion column to remove endogenous glucose from samples in lieu of a dialysis or protein-precipitation step might simplify and speed up the test, but this alternative has not been reported in the literature. The interassay CVs that have been reported, 1.2–13%, indicate that even as a manual procedure the TBA assay is capable of good precision. However, the TBA procedure appears to have been by-and-large supplanted by the nitroblue tetrazolium assay.

Two variants of the TBA procedure deserve mention. Lenzi et al. (88) have measured the TBA reaction product by using "high-performance" liquid chromatography rather than colorimetry. Their reversed-phase system uses a sodium acetate (5 g/L, pH 4.3) mobile phase and ultraviolet detection (280 nm). Little et al. (89) have developed a method for estimating glycated whole-blood proteins (hemoglobin and plasma proteins) by using capillary blood collected and stored on filter paper. This novel approach is primarily a measure of glycated hemoglobin and involves use of a TBA method originally designed for analysis of glycated hemoglobin (90).

Nitroblue Tetrazolium (NBT) Colorimetric Procedure

The NBT method, devised by Johnson et al. (2), is based upon the reducing ability of fructosamines in alkaline solution (91). Under alkaline conditions, Amadori rear-

rangement products such as fructosamines have reducing activities that can be differentiated from those of other reducing substances such as glucose and N-glucosylamine derivatives of labile Schiff bases (3, 7–9). In the NBT assay, a serum sample is added to carbonate buffer (at basic pH, originally pH 10.8, and 37 °C) containing NBT, which is subsequently reduced; the absorbance at 530 nm is measured 10 and 15 min later (2). The exact mechanism of the reduction of NBT by fructosamine is not known, but recently Jones et al. (92) have presented evidence that it involves a superoxide radical intermediate. 1-Deoxy-1-morpholinofructose (DMF, a synthetic ketoamine) in a 40 g/L solution of albumin was originally used for a standard. The 10-min incubation is necessary to allow fast-reacting interfering reducing substances to react. Because glucose reduces NBT at a pH above 11, but does not react between pH 10.5–11, there is no need to remove endogenous glucose from patients' specimens. However, albumin does contribute to the absorbance at 530 nm. The NBT assay was quickly adapted to a bichromatic discrete analyzer, demonstrating its potential as an automated test (93).

When investigators noted that activity measured with the NBT assay changed with buffer pH, the buffer pH was subsequently lowered to 10.35 from 10.8, which significantly increased the reference range for fructosamine in diabetic and nondiabetic subjects (94–98). The change in buffer pH was apparently abetted by the introduction of a commercial fructosamine assay kit (F. Hoffmann-La Roche, Basle, Switzerland), in which pH 10.35 is used and one corrects for the fructosamine activity due to the albumin in the standard.

The next modification of the assay was the development of glycated protein secondary standards in a bovine albumin matrix (40 g/L) instead of a DMF primary standard in human serum albumin (99). The secondary standards proved to be less variable with changes in reaction conditions, resulting in greater accuracy and better reproducibility. San-Gil et al. (100, 101), observing that reducing activities of human serum-based DMF standards differed from those of albumin-based standards, recommended the use of serum-based standards. However, this position has been disputed by Johnson and Baker (102), who found that the ketoamines in serum have five to six times the reducing activity of albumin-based DMF standards. This observation explains why NBT fructosamine values in serum are greater than both the expected concentration of glycated proteins and the concentrations measured in serum by other glycated protein assays (e.g., TBA, furosine) (103). Hindle et al. (104), studying DMF standards prepared in three different human albumin solutions at various protein concentrations, observed that the absorbance changes varied with the source of the albumin and the concentration used in the standards, but noted that the differences are not a major problem if a laboratory is cognizant of the characteristics of the standard it chooses to use and establishes its own reference range. Smid et al. (105) also found that DMF standards of equal concentration but prepared from different batches of human albumin exhibited different changes in absorbance. They recommended use of a DMF standard prepared in a human serum pool, because the calibration curves were parallel and absorbance differences similar for DMF calibrators prepared in various serum pools. Howey et al. (106) developed an internal standardization technique in which patients' samples are run twice, once without and once with added DMF, to minimize standardization and matrix effects.

It has been emphasized that fructosamine values will be falsely increased if a pre-incubation time of less than 10 min is used, owing to the presence of interfering substances in serum that reduce NBT during the first 10 min of the assay (103, 104, 107). However, the assay has been adapted to analyzers in which incubation times are only 7–8 min; performance is claimed to be acceptable, allowing for instrument-specific reference ranges (106, 108, 109).

The TBA assay has been used to measure glycated albumin, total glycated protein, or both, whereas the NBT assay was originally designed to measure total glycated proteins and not glycated albumin alone. Walker et al. (110) purified albumin from diabetic patients and found that the concentration of glycated albumin was correlated with the concentration of total glycated plasma protein. Albumin accounts for about 50% of total plasma protein; apparently, it is not necessary to measure glycated albumin specifically because total plasma fructosamine reflects glycated albumin, and both correlate with the average concentrations of blood glucose over the preceding intermediate-length interval of time.

The NBT assay has been adapted to a wide variety of automated chemistry analyzers, including: Cobas Bio, Cobas FARA, Cobas Mira, Hitachi 405, Hitachi 705, Abbott ABA-100, Abbott ABA-VP, Worthington Chemetrics, Technicon RA-1000, IL Monarch, IL Multistat 3, Rotachem II, Baker Encore, CentrifiChem 300, and CentrifiChem 400 (92, 99, 106, 108, 111–113). It has also been adapted to a continuous-flow analysis system (114).

Within-run and day-to-day CVs reported in the literature are very acceptable, typically in the range 2–7%, and an interlaboratory CV of 5.4% has been reported for a six-month study of 33 laboratories in which five different analyzers and the manual procedure were used (112). Using the Cobas Bio, Baker et al. examined 28 potential interferents; interference was notable only from bilirubin, EDTA, heparin, cysteine, and urate (99). When the same interferents were tested in the RA-1000 version of this procedure, heparin, bilirubin, hemoglobin, cysteine, and glutathione interfered, indicating that interference problems vary from analyzer to analyzer (115). Hyperlipidemia is common in diabetes, particularly in Type II diabetes, but hypertriglyceridemia has been reported not to interfere significantly with the validity of fructosamine measurements in diabetic subjects (116). The occurrence of in vitro glycation after the sample has been obtained does not appear to be a problem with the NBT assay, at least when specimens are kept for 24 h at 20 °C with a glucose concentration of 24 mmol/L (117). Acetylsalicylic acid reportedly significantly inhibits the in vitro glycation of albumin and other proteins and may pose a problem in vivo (118). Superoxide dismutase in serum is a potential interferent, causing falsely low values, but its concentration is ordinarily too low to be a problem (92).

The NBT method is attractive because it is fast, reproducible, cheap (in terms of the reagents, at least), and easily automated. At present, the major analytical concerns with the assay are the effect of the serum albumin concentration on fructosamine values and how best to calibrate the procedure. Some groups have found that hypoalbuminemia influences the assay but that the fructosamine concentration is independent of the albumin concentration when the latter exceeds 30–35 g/L (94, 119, 120). Van Dieijen-Visser et al. (121), however, reported that fructosamine values are affected by the albumin concentration whether it is greater or less than 30 g/L. They suggest adjusting the fructosamine value by subtracting 0.023 mmol of fructosamine per gram

of albumin per liter. Howey et al. (106) and Mosca et al. (113) have also found fructosamine to vary with albumin and total protein. Howey et al. propose the following correction: fructosamine (mmol/L) – 0.03 albumin (g/L) + 0.9 (mmol/L). Although Lim and Staley (120) did not find fructosamine to be significantly influenced by the albumin concentration, they did warn that increased protein catabolism, leading to a decreased half-life of protein, might cause the fructosamine concentration to reflect the mean glucose concentration in blood over a period of less than the roughly 21 days expected. Lloyd and Marples (122) measured fructosamine in thyrotoxic and hypothyroid patients, in whom protein turnover was increased and decreased, respectively; fructosamine was significantly decreased ($P < 0.001$) in the thyrotoxic patients and increased ($P < 0.05$) in the hypothyroid patients. Thus investigators must exercise caution in interpreting fructosamine results in patients with an abnormal rate of serum protein turnover.

Baker et al. (99) originally used DMF in a 40 g/L human albumin solution for standardization of the assay but later changed to secondary standards of glycated bovine albumin (40 g/L in isotonic saline), which had been calibrated by using the DMF/human albumin primary standard. As noted previously, San-Gil et al. (100, 101) and Smid et al. (105) recommend the use of DMF standards prepared from pooled human serum. Lever et al. (123) have suggested that the development of better primary calibrants deserves as much emphasis as developing new methods for the assay of fructosamine—a practical suggestion with much merit.

One final modification of the NBT assay bears mention. Caines et al. (124) measured glycated albumin with the Electro-Nucleonics Flexigem centrifugal analyzer, using the NBT assay but substituting 3-(4,5-dimethylthiazol-2-yl)-2,4-diphenyltetrazolium bromide (MTT) for NBT. MTT gave a threefold greater increase in absorbance than did NBT.

Clinical Applications

Protein Glycation in Diabetes Mellitus

A variety of proteins are subject to nonenzymatic glycation. The amount of glycated protein is increased in diabetes mellitus and is thought to contribute to the long-term complications of the disease (125–129), e.g., through enzyme inactivation, inhibition of binding of regulatory molecules, decreased proteolysis, and the crosslinking of glycated proteins and the trapping of soluble proteins by glycated proteins (129). Glycated proteins (summarized in Table 1) are typically increased in diabetes. The concentrations of glycoproteins, such as haptoglobin and fibrinogen, are also increased in diabetes, but glycoprotein synthesis results from the activity of glycosyltransferases and should not be confused with synthesis of fructosamines, which result from nonenzymatic glycation (130–138).

Measurement of glycated hemoglobin, Hb A_{1c}, has proven to be particularly useful in monitoring the effectiveness of therapy in diabetes, reflecting the long-term (six to eight weeks) control of glucose concentrations in blood (125, 126, 139). Hb A_{1c} is widely used as a marker of metabolic control in the diabetic patient, reflecting the effectiveness of the combined therapy of physical activity, diet, and insulin or other pharmacological agents. The traditional determination of glucose in blood indicates the status of glucose control at a given point in time, but only the continuous monitoring of glucose can give an accurate picture of the blood sugar status of a diabetic patient over an extended period. Continuous monitoring and adjustment of blood glucose being at present impractical, or unnecessary, for the majority of the diabetic population, Hb A_{1c} is a useful test for long-term monitoring. Of what value is fructosamine analysis for monitoring diabetic therapy over an extended period?

Clinical Usefulness of Fructosamine Analysis

Fructosamine as determined by the TBA method is clearly increased in diabetics as compared with nondiabetic controls (59, 60, 64, 79, 81, 85, 140, 141). In some cases, investigators have observed no overlap between the two groups; in other cases, the overlap was only slight. Similar observations have been made when the NBT assay was used (2, 95, 119, 142). Lim and Staley (111) found no significant differences among ambulatory patients, nondiabetic antenatal patients, and hospitalized patients, but values for all three groups differed ($P < 0.01$) from those for a population of insulin-dependent and non-insulin-dependent diabetic subjects by the NBT method. Two groups (143, 144) have challenged the effectiveness of the NBT assay in distinguishing normals from diabetics; however, the weight of the evidence in the literature overwhelmingly shows that, if used properly, the NBT fructosamine test is useful for monitoring diabetes. Fructosamine determinations by affinity chromatography likewise distinguish normal and well-controlled subjects from poorly controlled individuals (58, 71, 72, 76). The furosine assay also differentiates insulin-dependent diabetic subjects from normal subjects (145).

Results by the furosine method correlate with both Hb A_{1a-c} ($r = 0.790$) and the mean concentration of blood glucose ($r = 0.878$) in diabetic patients (145). The affinity chromatography method likewise correlates with Hb A_{1c} measurements (73). Several studies involving the TBA method show that fructosamine correlates with the concentrations of glucose and Hb A_{1c} in blood, although in some cases the correlation with fasting blood sugar in Type I diabetic subjects was not impressive (64, 66, 79, 81, 146–150). Similarly, numerous groups have found that the NBT assay correlates not only with the concentrations of blood glucose and glycated hemoglobin but also with glycated protein as determined by the TBA and affinity chromatography methods (2, 95, 96, 99–104, 107–111, 119, 151).

How does fructosamine analysis compare to the measurement of blood glucose and glycated hemoglobin in terms of clinical usefulness? The half-life of hemoglobin is about 60 days, that of albumin about 14–20 days, and that of various other proteins, 2.5–23 days (59, 147, 148). Fructosamine reflects the mean glucose concentration in blood over a period of about two to three weeks while the Hb A_{1c} concentration is indicative of the concentration of blood sugar over a six- to eight-week period. In a study of rabbits made diabetic by administration of alloxan, Rendell et al. (76), using affinity chromatography, found that the concentration of glycated albumin plateaued four weeks after alloxan administration, while concentrations of glycated hemoglobin were still rising. This same group found that in a group of diabetic patients being brought into better glycemic control, the concentrations of glycated albumin entered the normal range and plateaued, while the concentrations of glycated hemoglobin were still falling. The conclusion is that fructosamine is a more responsive marker of average blood sugar values than is glycated hemoglobin. Using the TBA assay, Jones et al. (152) noted that preprandial blood sugar and fructosamine results decreased by 72% and 58%, respectively, after the initiation of therapy in

newly diagnosed diabetic subjects, but Hb A_1 fell by only 39%; after eight weeks there were no significant differences in the percentages of decrease of the three parameters. Kennedy et al. (146, and cited in 153) reported that fructosamine values dropped 37% after one week of improved treatment in a group of poorly controlled diabetic subjects, while Hb A_{1c} decreased only 8%. Day et al. (154) found that when insulin was withdrawn from diabetic rats, the half-times to reach new steady-state concentrations of glucose, fructosamine, and glycated hemoglobin were about two, three, and eight days, respectively. When insulin therapy was reinstituted, the half-times to return to baseline values were two, 3.5, and 15 days, respectively. Shin et al. (155) studied the concentrations of glucose, glycated hemoglobin, and glycated albumin in plasma of fairly well managed and poorly managed diabetics and observed the greatest absolute changes in the concentration of glycated albumin. Kennedy and Merimee (156) found that fructosamine accurately reflects the alteration of blood sugar concentrations one to two weeks after improvement of glycemic control in diabetics, whereas Hb A_1 takes up to eight weeks to correlate with the altered degree of control. Dolhofer and Wieland (64) observed Hb A_{1a-c} to decrease by only 15% after 20 days of improved therapy in a diabetic patient, while fructosamine decreased by more than 50% during the same time.

Using the NBT assay, Baker et al. (157) found fructosamine to be more sensitive than determination of either Hb A_{1c}, 24-h urinary glucose, or fasting blood glucose in detecting deterioration of glycemic control after the withdrawal of oral hypoglycemic agents in Type II diabetes. Ross et al. (158) found the NBT assay to be superior to glycated hemoglobin and the TBA assay in distinguishing between four groups of diabetic patients (well-controlled, moderately well-controlled, poorly controlled, and newly diagnosed). Because the within-day individual variability of fructosamine has been found to be 4.1%, with no significant effect from postprandial blood glucose concentrations, random sampling is sufficient (95, 96). Even experienced physicians have difficulty assessing diabetes control, with a tendency to underestimate the mean concentration of blood glucose when hyperglycemia is present, but fructosamine determination correctly classified glycemia control in most cases (96).

What advantage does the determination of fructosamine have over the assay of glycated hemoglobin? Basically, because the half-life of albumin and the other serum proteins is considerably shorter than that of hemoglobin, the concentrations of fructosamine will change more rapidly than those of glycated hemoglobin. Fructosamine thus serves as an index of intermediate-term diabetic control (one to three weeks) that can alert the physician to deteriorating control before changes in glycated hemoglobin can be detected—which may not be apparent for at least four weeks after improvement of glycemic control (119, 146). In anemia, hemolytic or otherwise, marked changes in the life span of hemoglobin molecules may make glycated hemoglobin values unreliable, whereas the blood proteins are relatively stable (75). Hemoglobinopathies or recent transfusion can also adversely affect the usefulness of glycated hemoglobin values (66). From the perspective of the clinical chemist, fructosamine assays, particularly the NBT assay, offer the advantages of being quick, technically simple, inexpensive, precise, fairly free of interferences and easily automated for use with microsample volumes (96, 151, 157). Of course,

fructosamine values are affected by low concentrations of albumin and plasma proteins, as in cases of protein-losing nephropathy or liver failure, and must be interpreted with caution under these circumstances (75, 111, 119, 122).

Besides being a measure of intermediate diabetic control, the fructosamine assay may even be useful as a screening test for diabetes and for managing diabetes of pregnancy. Baker et al. (119) found the NBT assay to have a 0.75 probability of a true diagnosis of diabetes, as compared with probabilities of 0.58 and 0.56 for measurement of glycosuria and a 2-h postprandial glucose, respectively, in 74 patients referred for an oral glucose tolerance test. Lloyd and Marples (94) found that the NBT test detected 25 (84%) of 30 untreated diabetics and yielded four false positives (8%) for 50 nondiabetic subjects. In contrast, Howey et al. (106) do not endorse the use of the NBT fructosamine assay as a screening test for diabetes because of the considerable intra- and interindividual variation of fructosamine they observed. Roberts et al. (93, 159) found fructosamine to be effective in detecting diabetes in pregnancy in two studies, one in which eight out of nine women with gestational diabetes had a fructosamine concentration greater than the 95th percentile and another in which the test detected 17 of 20 women with gestational diabetes (85%) and gave 5% false positives for 79 nondiabetic women. In a study of 14 insulin-dependent diabetic women, fructosamine concentrations decreased by 50% after four weeks of good diabetic control, whereas glycated hemoglobin did not show a comparable improvement until after 12 weeks (74).

How the fructosamine assay will eventually fit into clinical practice is not yet clear. One group, after comparing Hb A_1, TBA, and NBT assays concluded that neither the TBA or NBT test should be substituted for glycated hemoglobin analysis but that a combination of fructosamine and Hb A_1 determinations is useful (160). Ordering fructosamine tests in conjunction with the glycated hemoglobin test is not unlikely as a complementary assay. The tests measure different molecules with different half-lives and therefore will not necessarily correlate under conditions of changing glycemic control (142). Fructosamine determination may not replace glycated hemoglobin analysis but certainly has the potential to become a routine test in clinical chemistry laboratories.

The Fructosamine Assay of Choice

Assuming that further clinical evaluations of the fructosamine assay prove it to be of value, either in conjunction with Hb A_{1c} or on its own, which of the several methods available is the best for the clinical laboratory? Goldstein et al. (21), in their recent review of glycated hemoglobin methodologies, concluded that each of the methods reviewed had its advantages and disadvantages, and that all provided useful clinical information "if performed properly." Presumably, the same can be said of the various fructosamine methods, but a few concluding comments are appropriate. The affinity chromatography and NBT assays are the likely candidates to become routine procedures. Although many studies have been published about the TBA method, it is clearly neither as simple nor as rapid as these two, and neither the phenylhydrazine derivative nor the furosine methods have been adequately developed yet. A fluorometric method for glycated hemoglobin developed by Gallop et al. (161) is applicable to assay of fructosamine but has not yet been adapted for that use; it, too, would require automation to make it a competitive method. Lloyd et al. (162) have

shown that glycated total protein as determined by the affinity chromatography method correlates well with that measured by the NBT assay ($r = 0.91$, $P < 0.001$) for both nondiabetic and diabetic patients. As noted, two commercial affinity column kits are available, which smaller clinical chemistry laboratories may find attractive. The NBT assay is available as a commercial kit from two manufacturers (from Roche, as previously mentioned; and "Glyco-Probe GSP," from Isolab). The NBT method is also readily adaptable to numerous automated analyzers, making it attractive to the larger clinical chemistry laboratories.

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